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RESEARCH PAPER

Effects of different Process Variables on Chemical Attributes of Yoghurt and its Feeding Impact of Albino Rats

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ABSTRACT

Yoghurt was prepared from fresh cow and buffalo milk with standardized fat content of 4% , 5% and 6%; SNF 10%, 11% and 12% along with 2% starter culture of *Streptococcus salivarious* subsp. *thermophilus* and *Lactobacillus delbruechii* subsp. *bulgaricus* in the ratio of 1:1, 1:2 1:3, 2:1 and 3:1; at 2 temperature levels of 39°C and 42°C for 6 hours. The acidity of yoghurt was higher in samples with higher levels of solid. Protein lactic and ash contents increased as the SNF contents increased in milk. The yoghurt based diet was fed to experimented albino rate. The levels of *S. thermophilus and L. bulgaricus* (3:1) acidity increased and pH were decreased in yoghurt. The levels of cholesterol decreased (P<0.01) from 61.59-50.80 mg/100 ml of blood when the levels of yoghurt increased and the triglycerides decreased (89.0 to 83.0 mg /100ml) as the levels of yoghurt increased (10 % - 30%) in the diet. The concentration of phospholipids in the blood increased (P<0.01) 27.27% in 10% yoghurt fed group and 13.64% to 27.27% in yoghurt fed group than the control.

Keywords: Albino rat, blood chemical, feed intake, temperature, yoghurt

Probiotics are defined as live microbial food ingredients that have a beneficial effect on human health (Salminen *et al.*, 1998). The concept of probiotics evolved at the turn of the 20th century from a hypothesis first proposed by Nobel Prize winning Russian scientist Elie Metchnikoff (Bibel, 1988), who suggested that the long, healthy life of Bulgarian peasants resulted from their consumption of fermented milk products.

Yoghurt is a probiotic lactic acid fermented product obtained by souring milk using a pure starter culture of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (FAO 1990). The role of these two genera in yoghurt manufacture is mainly identified as milk acidification and synthesis of aromatic compounds (Sahan *et al.*, 2008; Serra *et al.*, 2009; Fadela *et al.*, 2009). Fermented milk products such as yoghurt were originally developed as a simple choice of preserving the nutritional goodness of milk. But soon it was realized that fermenting the milk with different microorganisms could also be an opportunity to develop a wide range of products with different flavors, textures, consistencies and several health attributes (Michelle, 2005).

Production and consumption of yoghurt is growing world over continuously due to its therapeutic properties, antibacterial activity and high nutritive value (Karagul *et al.*, 2001 and He *et al.*, 2005). There are several reports on the beneficial effects of yogurt on the human health and several research findings have suggested that the consumption of fermented dairy products, including yogurt, elicits anti-tumor

effects (Hosono *et al.*, 1986; Wollowski *et al.*, 1999; Zsivkovits *et al.*, 2003). Yoghurt is also reported to have other health promoting properties like preventing gastrointestinal upsets, lowering blood cholesterol due to presence of live acid producing bacteria (Schieber *et al.*, 2002; Marona and Pedrigon, 2004; Doornbos *et al.*, 2006). Hargrove and Alford (1978) demonstrated that supplement of yoghurt in the diets improves the growth rates of rat. The growth effect and therapeutic reputation of yoghurt's prompted the present study.

The aim of the present work was to assess the hypocholesterolaemic potential of yoghurt standard and yogurts fortified with skim milk powder, levels of fat and starter culture strength, using albino rats as an animal model.

MATERIALS AND METHODS

Raw materials

Milk samples were procured from Banaras Hindu University Dairy Farm during July 2007 to November 2009, Varanasi. The freeze dried pure culture of *Streptococcus salivarious subsp. thermophilus* and *Lactobacillus delbruechii subsp. bulgaricus* procured from the National Dairy Research Institute, Karnal, Haryana (India).

Yoghurt

Five hundred forty samples of Yoghurt were prepared from fresh cow (M_1) and buffalo (M_2) milk standardized at 3 levels of fat 4% (F_1), 5% ($F_{2)}$ and 6%

Table 1: Interaction effect of temperature, milk, fat and starter culture on chemical attributes of yoghurt

Factors		Chemical attributes (average)					
		Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Acidity (%) (Lactic Acid)	pH
Sources of Milk	M_1	5.02	4.72	5.19	1.02	0.97	4.55
	M_2	5.02	4.72	5.32	1.02	0.98	4.54
	CD 5%	—	—	0.043	—	0.004	0.007
	1%	—	—	0.057	—	0.005	0.009
Temperature	T_1	5.02	4.72	5.32	1.02	0.96	4.57
	T_2	5.02	4.72	5.19	1.02	1.00	4.52
	CD 5%	—	—	0.043	—	0.004	0.007
	1%	—	—	0.057	—	0.005	0.009
Fat	F_1	4.02	4.71	5.26	1.02	0.98	4.55
	F_2	5.02	4.72	5.28	1.02	0.98	4.55
	F_3	6.02	4.72	5.22	1.02	0.98	4.54
	CD 5%	0.018	—	—	—	—	0.008
	1%	0.023	—	—	—	—	—
SNF	S_1	5.02	4.29	4.81	0.88	0.94	4.60
	S_2	5.03	4.67	5.25	0.97	0.98	4.53
	S_3	5.02	5.19	5.70	1.20	1.01	4.51
	CD 5%	—	0.020	0.053	0.014	0.004	0.008
	1%	—	0.027	0.069	0.018	0.006	0.011
Starter Culture	Sat_1	5.03	4.71	5.19	1.01	0.89	4.63
	Sat_2	5.01	4.73	5.38	1.01	0.90	4.61
	Sat_3	5.02	4.71	5.56	1.01	0.92	4.59
	Sat_4	5.03	4.72	5.13	1.03	1.08	4.47
	Sat_5	5.01	4.72	5.01	1.02	1.11	4.44
	CD 5%	—	—	0.068	—	0.006	0.011
	1%	—	—	0.069	—	0.008	0.014

n=3

(F_3); 3 levels of solid-not-fat (SNF) 10% (S_1), 11% ($S_{2)}$ and 12% (S_3) along with 5 levels of starter culture of *Streptococcus salivarious subsp. thermophilus* (S) and *Lactobacillus delbruechii subsp. bulgaricus* (L) in the ratio of 1:1 (Set_1), 1:2 ($Set_{2),}$ 1:3 (Set_3), 2:1 (Set_4) and 3:1 (Set_5) at 2 temperature levels 39^0C (T_1) and 42°C (T_2). Fat and SNF levels, were adjusted in the milk using skim milk and fresh milk fat. Before adding starter, cultured milk was heated at 90°C for 10 min and cooled at 40 °C. The levels of fat were adjusted with the addition or deletion of cow milk fat in cow milk samples and buffalo milk fat in buffalo milk samples separately, 2% starter cultures were added in each milk sample maintained at respective temperatures.

Analysis after 6 hours of incubation, all the samples were chemically examined for fat, protein, lactose, ash and acidity by the procedures given in (AOAC, 2000). The data were statistically analysed as per technique (Factorial-Randomized Block Design) described by Snedecor and Cochran (1967).

Feeding Albino Rats

Ranging in weight from 37- 45 gm albino rats of similar body weight and body conformation were selected at their one month of age and randomly divided into 5 groups of six rats in each. The basal diet having 15% protein was prepared by mixing ground maize, oat, wheat and soybean seeds in the ratio of 3:1 : 2:1. The best quality of yoghurt prepared from buffalo milk of 6% fat, 12% SNF with culture Set 3 at 42 °C was selected for the feeding of rats. The rats were fed on basic ration (group A) along with 10% buffalo milk of 6% fat and 12% SNF (group B); 10% yoghurt (group C) ; 20% yoghurt (group D); and 30 % yoghurt (group E), twice daily. Fresh and clean drinking water was offered in feeding bottle to all the rats. The rats were weighed at every 4th days of intervals. At 32 days of experiment, the blood samples were collected separately from jugular vein of each rat in the sterlized sample tubes. Serum cholesterol and sugar levels were estimated by calorimetric method using O-phthalaldeyde procedure given by Ruddel and Moris (1973).

Statistically analysis

The data were statistically analysed as per significance of results were listed at Kog i.e. P<0.01 method described by Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Effects on chemical attributes

The lactose (5.32%) and acidity (0.98%) in buffalo milk yoghurt were significantly higher than the values observed in cow milk yoghurt (5.19% and 0.97%, respectively). The pH of cow milk yoghurt (4.54) was higher than buffalo milk (4.55) yoghurt (Table 1). The lactose content (5.19%) and pH (4.52) in yoghurt prepared at higher temperature were significantly lower than the lactose (5.32%) and pH

Table 2: Effect of yoghurt feeding on the performance of albino rat

Groups / Treatments	Feed intake/ head/day (g)	Gain in body weight/head/day (g)	Blood cholesterol (mg/100 ml)	Blood sugar (mg/100 ml)	Triglycerides (mg/dl)	Phospholipids (mg/100ml)
Group A	34.29	1.65	61.59	48.54	86.00	6.82
Group B	37.86	2.06	56.50	81.06	89.00	8.68
Group C	41.52	2.01	53.10	87.57	88.00	7.75
Group D	43.85	2.05	51.97	87.37	85.00	8.68
Group E	51.66	2.23	50.80	86.30	83.00	8.06
CD 5%	2.46	—	0.71	2.09	2.34	0.24
1%	3.32	—	1.02	3.05	3.41	0.35

n=6

(4.57) of yoghurt prepared at lower temperature. With increasing levels of SNF in yoghurt, the acidity per cent increased significantly. Similarly higher acidity and lower pH have been reported by (Ahmed, 1992), when zabadi was prepared with higher coco milk containing more SNF. The levels of fat in yoghurt increased with increased levels of fat in milk. The other components of yoghurt did not differ significantly, except pH which decreased at highest level of fat. The average lactic acid content increased and pH of yoghurt decreased as the proportions of S*treptococcus salivarous subsp. thermophilus* and *Lactobacillus delburechii subsp. bulgaricus* increased in the milk. The greater acidity (P<0.01) in starter Set_4 and Set_5 were possibly due to domination of S*treptococcus salivarous subsp. thermophilus* in the mixed culture. The acidity of yoghurt was higher in the sample prepared with higher levels of total solids. The similar observation has also been reported by Humphreys and Plunkett (1969) and Patel *et al.* (2009).

Protein, lactose and ash contents of yoghurt increased (P<0.01) as the concentration of SNF increased in the milk. The increases of these components in yoghurt were due to presence of higher amount of chemical components in the SNF.

Effects of feeding yoghurt on albino rats

The feed intake in rats increased ((P<0.01) when diet was supplemented with 10% milk or yoghurt than the control (Table 2). The increase in feed intake in yoghurt feed group C was 9.67% higher that of group B and 21.08% higher than that of group A. The feed intake increased in all the groups when the levels of yoghurt were increased in the diet. The voluntary food intake is a function of the hypothalamic control of feed intake to large extent, as has been the case in present study. (Hitchins *et al.,* 1983) have reported that stimulation of weaning rat growth by yoghurt and related fermented milk is associated with increase feed consumption and increased efficiency. The gain in body weight increased as the levels of yoghurt was increased in the diet. These results corroborates with the finding reported by (Hargrove and Alford, 1980), where they noted better growth rate and feed efficiency when fermented products were fed to rats.

The blood cholesterol decreased (6.34%) when 10% milk was supplemented in rat feed. The levels of cholesterol decreased from 61.59 mg. to 50.80 mg. per hundred ml. of blood when the levels of yoghurt increased in the diet. This decrease may be due to lactic acid producing bacteria which may have directly bound the cholesterol into the small intestine before absorption or by deconjugating bile acid to produce free bile acid (Usma, 2000). Grunewald and Mitchel (1983); Rossi *et al.* (2008) have reported that acidophil milk had no effect on serum cholesterol in mice. The level of blood sugar increased (from 48.54 to 81.06 mg/100 ml) when yoghurt was added in the feed, but the differences in the values obtained at different levels (10% to 30%) of their inclusion in the feed were not significant. The level of blood sugar in milk as well as yoghurt fed groups were significantly (P<0.01) higher than the control. The higher concentration of blood sugar might have been the result of higher intake of diet with higher calories / gram by the rats. The level of triglycerides decreased (from 89.0 to 83.0 mg/100 ml) as the levels of yoghurt increased (from 10 % to 30%) in the diet. A small but no significant decline in triglycerides was also observed by (Akalin *et al.,* 1997) in mice having acidophilus diet. The concentration of phospholipids in the blood increased to 27.27% in 10% milk fed group and 13.64% to 27.27% in yoghurt fed group than the control (6.82mg / 100 ml).

CONCLUSION

Yoghurt of best quality characteristics can be prepared at 42°C with buffalo milk having 6% fat, 12% SNF using *L. delbruchi* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus* culture in 1:3 ratio. It is highly acceptable and nutritionally rich food which can be supplemented in daily diet of human being. It reduces the level of cholesterol as a food supplements in diet. It also reduces the level of cholesterol in the persons suffering from hypercholesterolemia.

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